THE THIRD QUALITY CONTROL MATERIALS FOR GENETIC TESTING MEETING

November, 9, 2004, Los Angeles, CA

SUMMARY

The third Quality Control Materials for Genetic Testing meeting, organized by the Centers for Disease Control and Prevention (CDC), was held on November 9, 2004, in Los Angeles, California. Participants of the meeting included over 50 experts in genetics and genomic testing from professional organizations, government agencies, industry, laboratories, academic institutions, proficiency testing (PT)/External Quality Assessment (EQA) programs, and cell repositories. The main goals of the meeting were to: 1) set priorities and goals for a sustainable process to provide QC materials needed by the genetics community based on the recommendations developed from the two previous meetings (September 2003 and March 2004) and additional input ; 2) obtain input on a CDC-based Genetic Testing Quality Control Materials Program (GTQC), which was proposed based on suggestions from the first two meetings, to coordinate and facilitate communication for QC material development, contribution, verification, and distribution; and 3) discuss approaches to sustain and expand the current efforts into a community process with defined steps to move forward.

The meeting began with an introduction and welcoming remarks by **Dr. D. Joe Boone**, Associate Director for Science, Division of Laboratory Systems (DLS), National Center for Health Marketing (NCHM), Coordinating Center for Health Information and Services (CoCHIS), CDC. The subsequent presentation sessions included a review of the two previous QC Materials for Genetic Testing meetings, project updates on the development of Standard Reference Materials (SRMs) for genetic tests by the National Institute of Standards and Technology (NIST), an update on CDC efforts to make QC materials available for genetic tests of public health significance, an overview of the Coriell Cell Repositories, a summary of issues and challenges in developing synthetic control materials, efforts to develop reference materials for genetic testing in Europe, and a presentation of the proposed GTQC Program. Two discussion sessions were held in the afternoon to seek suggestions for methods and ideas to make this program a sustainable community process and to develop strategies to continuously monitor the needs of the genetic testing community.

Introduction

The meeting began with welcoming remarks and charge to the group by Dr. Boone, followed by self-introduction by participants around the table.

Updates:

Review of Previous QC Materials Meetings, Future Challenges and Needs

Dr. Lawrence M. Silverman, Professor of Pathology at the University of Virginia, reviewed the progress that has been made through the first two QC materials meetings, which were held on Sept. 15-16, 2003 and March 8, 2004. He summarized the recommendations developed by the workgroups addressing the identified areas of need, including: 1) developing a scheme to set priorities for current and future needs; 2) developing networks of material contributors to facilitate participation and material collection; 3) developing processes to use existing cell banks as material sources; 4) identifying research activities on QC material development and facilitating collaboration among research efforts; 5) developing validation processes for QC materials; 6) developing professional guidance on appropriate use of QC materials; 7) clarifying regulatory oversight for providers and users of QC materials; and 8) developing better coordination of funding sources and opportunities. Dr. Silverman also highlighted several areas of molecular diagnostics in pressing need of appropriate QC materials, including molecular oncology testing and CGG repeat sizing for fragile X syndrome.

Development of SRMs for Fragile X CGG Repeats and Mitochondrial DNA Mutation Analysis

Drs. Kristy Richie and Barbara Levin of NIST discussed their work to develop SRMs for fragile X CGG repeat sizing and mitochondrial DNA mutation analysis, respectively. Dr. Richie described the development of a fragile X SRM to be used for the accurate determination of the CGG repeats in the FMR1 gene in the normal and pre-mutation range. This SRM (2399), which has been verified both at NIST and through inter laboratory evaluation, contains 9 different repeat sizes, ranging from 20 to 118 repeats. Subsequently, Dr. Levin discussed her work developing SRMs for evaluating mitochondrial DNA mutations and heteroplasmy determination for a number of mitochondrial disorders.

Development of Characterized Cell Lines as QC Materials

Dr. Laurina Williams, DLS, NCHM, CoCHIS, CDC, described the role of CDC in the development of positive control materials for genetic testing through a series of projects beginning in 1999. She then described in detail the CDC project that developed 40 cell lines for QC use, including 27 cell lines derived from residual patient blood specimens, 6 from cell lines previously deposited in the Coriell Cell Repositories, and 7 negative control cell lines. Twenty-seven cell lines containing mutations previously unavailable to the public, including mutations associated with connexin 26-associated deafness, Muenke syndrome, cystic fibrosis (CF), fragile X syndrome, HFE-associated hereditary hemochromatosis, Huntington disease, MTHFR and alpha-thalassemia, have been reference and pilot tested and verified in multiple laboratories using at least two different testing methodologies. These cell lines are now available from Coriell Cell Repositories.

Summary of the Rare Disease Testing Conference

Dr. Joe Boone presented a summary of the "**Promoting Quality Laboratory Testing for Rare Diseases: Keys to Ensuring Quality Genetic Testing**" conference on May 19-21, 2004, in Atlanta, GA. This conference was a collaborative effort between the CDC, the Office of Rare Diseases of the National Institutes of Health, Emory University, the American Society for Human Genetics (ASHG), the American College of Medical Genetics (ACMG), the Health Resources and Services Administration (HRSA), and the Genetic Alliance. Dr. Boone summarized the major recommendations developed at this meeting and immediate outcomes, including: 1) the formation of the North American Rare Disease Laboratory Network; 2) the commitment of ASHG and the Office for Human Research Protections, HHS, to provide education for researchers and IRBs about the importance of patient testing in CLIA laboratories; 3) expansion of an NIH pilot program to fund translation of potential tests from the research phase into clinical settings; and 4) the plan to have a follow-up meeting in 2005 to develop a process for integrating the recommendations into action.

Overview of the Coriell Cell Repository

Dr. Jeanne Beck, Director, Coriell Cell Repositories, presented an overview of the Coriell Cell Repository, including the variety of cell collections, diseases for which characterized mutations are available, and the specimen submission process. She described approaches by which Coriell assesses the needs of the community, including analyzing unsuccessful online searches, monitoring the volume of shipments of biomaterials, and conducting user surveys at major genetics meetings. Based on Coriell data, cystic fibrosis and fragile X represent the disorders with the largest number of shipments. There has been a steady increase in the shipments of cystic fibrosis DNA samples over the last 10 years, especially since late 2001 after the implementation of the recommendations by the American College of Obstetricians and Gynecologists (ACOG) and ACMG for preconception and prenatal CF carrier screening. Dr. Beck summarized results of surveys Coriell conducted at five major genetic meetings, including the 2001 meeting of the International Congress of Human Genetics, the 2002 and 2004 annual meetings of the European Society of Human Genetics, and the 2002 and 2004 annual meetings of ASHG, to capture QC materials needs by the US and international genetics communities. Among the 21 disorders listed in the survey, fragile X syndrome, cystic fibrosis, BRCA1 and BRCA2 hereditary breast/ovarian cancer, Factor V Leiden thrombophilia, and HFE-associated hereditary hemochromatosis represented the diseases having the most requests for characterized mutations.

Development of Synthetic DNA Control Materials

Dr. Bassem Bejjani, Co-Director Molecular Diagnostics Laboratory, Sacred Heart Medical Center, Spokane, WA, described the development of synthetic QC materials with multiple CF mutations that can be assayed in a single reaction well. Dr. Bejjani highlighted issues and challenges in developing synthetic control materials by a

laboratory that primarily provides clinical genetic testing, including 1) determining the types of control materials needed to meet the requirements and expectations for the tests; 2) cost concerns in devoting staff time and resources to such efforts; 3) the regulatory requirements that have to be met for providing the materials to other laboratories. Dr. Bejjani indicated that the synthetic control materials for CF mutation analysis developed in his laboratory have been pilot-tested in a number of reference laboratories using a variety of testing methodologies. He proposed that the using synthetic controls in conjunction with genomic controls would have advantages of reducing test costs and providing positive controls simultaneously for all the mutations to be detected in an assay.

The Eurogentest Project

Dr. Els Dequeker, University of Leuven, Leuven, Belgium, gave a presentation about the Eurogentest project (Genetic Testing in Europe – Network for Test Development Harmonization, Validation and Standardization of Services). This project, which begins in January 2005, will work to improve the quality and standardization of genetic testing across Europe. The project is divided into 6 working units: 1) Quality Management and Accreditation/Certification of Genetic Testing; 2) Information Sources and Bio-informatics tools; 3) Clinical Genetics, Community Genetics and Public Health; 4) Ethical, Legal, Social lssues; 5) Research and Emerging Technologies; and 6) Education and Information. The focus of Working Unit 1, Quality Management and Accreditation/Certification of Genetic Testing, chaired by Drs. Dequeker and Michael Morris, is to develop QC and certified reference materials (CRMs) and to explore procedures to improve the quality of genetic testing in Europe.

The CRMGEN project

Dr. David Barton, Coordinator, CRMGEN Project, Irish National Centre for Medical Genetics, Our Lady's Hospital for Sick Children Dublin, Ireland, presented an update on CRMGEN, a European project to develop CRMs for genetic tests. The goal of this project is to generate prototype reference materials and to conduct validation studies necessary for the production of CRMs for a range of molecular genetic tests. Currently, reference materials are being developed for cystic fibrosis, hereditary haemochromatosis, fragile X syndrome, sickle cell anemia, beta thalassemia, factor V thrombophilia, hereditary non-polyposis colon cancer, and Duchenne muscular dystrophy.

The Genetic Testing Quality Control Materials Program (GTQC)

Dr. Bin Chen, DLS, NCHM, CoCHIS, CDC, provided an overview of the purposes and major activity areas of the proposed CDC-based GTQC Program, which was developed based on recommendations from the previous working meetings. The purposes of this program are to 1) assist the genetic testing community in obtaining appropriate and validated materials for QC, PT, test development, and research, and 2) to facilitate information exchange, communication, and coordination for contribution, development, validation, and distribution of materials for genetic testing. Major activities of the GTQC

Program include: 1) coordination of information exchange among users, providers, and developers of QC materials through an interactive website; 2) monitoring and assessment of the needs of the genetic testing community; 3) facilitating the submission, development, and validation of QC materials; and 4) development of a sustainable community process through collaborations and partnerships.

Dr. Lisa Kalman, DLS, NCHM, CoCHIS, CDC, who will serve as the first QC Materials Coordinator (QCMC), presented the proposed project plan. She provided a preview of an interactive website, to be publicly launched early 2005, which will facilitate information exchange about QC material needs and availability. The website homepage will have a description of the project, a user bulletin board for communication between users of QC materials and a "contact us" link. Within the website, six pages have been designed to provide 1) information about available QC materials and their verification status, 2) a list of QC materials that are currently needed by the community, 3) information to assist QC material contributors, 4) links to professional, practice and regulatory guidelines, 5) reference articles, and 6) information about QC material funding and incentives. Once the community QC needs have been identified and prioritized, the QCMC will work to improve public availability of QC materials by locating potential sources of the materials and coordinating contribution to public repositories. The QCMC will also coordinate verification studies by genetic testing laboratories. In addition, the QCMC will be responsible for updating the inventory lists on the QCMCP website and informing the genetics testing community on a timely basis.

Dr. Kalman also provided a description of a new collaborative effort with Drs. Stephanie Sherman and Steve Warren of Emory University, who have offered to donate fragile X cell lines with characterized CGG repeat sizes to the Coriell Cell Repositories. The CGG repeat sizes and other characteristics of each cell line will be verified through inter-laboratory studies and the materials will be made available to the community.

Group Discussion

Discussion 1 – Establish a Community Process and Move Forward

This discussion session was moderated by Drs. Joe Boone and Bin Chen. The group discussed approaches to make the GTQC Program a sustainable community process, through leadership, support and/or participation of existing resources, professional organizations, and laboratories. The formation of an Expert Panel to provide advice and input on both the GTQC Program activities and website was also discussed. After considering the proposed activity areas and input needed from the community, participants provided the following comments:

Expertise Needed for the Expert Panel

Participants expressed strong enthusiasm about the GTQC Program and were in agreement that this program represented a significant step forward in sustaining the process to make QC materials available to the community. The need for an Expert Panel to provide input and advice on both the activities and the website of the GTQC Program was strongly supported by the participants. Among the suggested representation the Expert Panel should include experts in various areas of genetic and molecular diagnostics, cell repositories and other providers of QC materials, GeneTests, relevant government programs in NIST and the Food and Drug Administration, experts on informed consent and human subjects protection issues, patient advocacy groups, and manufacturers of *in vitro* diagnostics and QC materials. It was recommended that initially, the Expert Panel should consist of these experts and leaders of the previous working subcommittees.

Issues Related to Submitting Patient Samples and Cell Lines to Cell Repositories

Participants discussed the process of submitting existing cell lines and residual patient specimens to cell banks for the purpose of providing QC materials to the community. Concern was expressed on whether this effort would constitute human subjects research and to what extent Institutional Review Boards (IRBs) should provide oversight for the protocols. Several participants commented on the difficulty they encountered in getting IRB exemption for their submission protocols. It was suggested that using patient materials for QC purposes should be considered clinical practice, not research; and use of completely anonymous specimens available from cell banks should be considered exempt under 45 CFR 46. On the other hand, it was recognized that IRBs also consider State, local and institutional requirements. For example, New York State requires patient consent for use of patient specimens as QC materials. It was agreed that informed consent issues and the role of IRBs should be clarified in order to support the process for facilitating contribution and public availability of materials for QC purposes. Experts were identified at the meeting to examine these issues and the available resources, to strategize approaches towards developing generalizable guidance for a community process to contribute needed materials for QC, PT/EQA, test validation, and test development purposes.

Discussion 2 – Needs of the Community and Strategies to Meet the Needs

This discussion was moderated by Dr. Jean Amos, Scientific Director, Molecular Genetics, Specialty Laboratories, Inc., Santa Monica, CA, and Dr. David Barton. Participants discussed tests and/or areas of testing for which materials are urgently needed and strategies to meet these needs.

QC Materials Needs for CF Testing

There was general agreement that the 25 mutations on the ACMG-recommended CF testing panel are now covered by cell lines currently available from the Coriell Cell Repositories as well as synthetic controls. However, unmet needs still exist for less

common mutations that are not on the ACMG panel, such as I507V, and mutations that are significant for specific subpopulations. It is also important to consider whether mutations in controls should be in a heterozygous or homozygous state and whether it is necessary to have homozygous cell lines for each mutation, recognizing that homozygosity is more easily accomplished in synthetic controls. It was suggested that needs for CF controls can be monitored through the ACMG/College of American Pathologists (CAP) Molecular Genetic Surveys, cell and material repositories, and the Expert Panel. It was also suggested that whole-genome amplification, might be a means to obtain control materials for rare CF alleles; however issues such as product quality and allele representation would need to be determined before considering this approach.

QC Materials Needs for Fragile X Testing

Participants discussed the technical challenges in precisely determining the number of CGG repeats in the FMR1 gene and the wide variation in repeat sizing among laboratories as reflected by CAP Molecular Genetic Survey results. In light of steadily increasing test volume for fragile X syndrome and the association of permutation alleles with adult-onset disorders, it was suggested that QC materials for standardizing fragile X CGG repeat sizing be considered as the highest priority for material development. The group then discussed the CGG repeat sizes needed as QC standards for fragile X testing. Among the suggested needs include:

- CGG repeat alleles in the normal range (5 – 44 repeats)
- Grey-zone or intermediate alleles, including those ranging from 45-54 repeats
- The upper limit of PCR-amplifiable alleles, usually around 90 repeats or above
- The upper limit of the premutation range (around 200 repeats)
- Repeats sizes around the cut-off for normal, grey-zone, and pre-mutation alleles
- Controls for partial methylation and mosaicism

It was suggested that fragile X alleles with adjacent repeat sizes (i.e. 48 +/- 1 or 90 +/- 2 repeats) should be developed and used to determine assay resolution. In addition, appropriate controls needed for the development of new-generation fragile X assays and potential assays for Fragile X-associated disorders should be considered.

Participants expressed enthusiasm about the CDC-Emory collaboration that will provide fragile X cell lines with characterized repeat sizes. A volunteer working group was formed to review the list of cell lines available from the Emory laboratories and select a subset that will potentially provide a comprehensive fragile X QC set, and to participate in inter-laboratory verification study once the cell lines have been expanded by the Coriell Cell Repositories. It was suggested that since expanded fragile X alleles are associated with replication instability, the stability of fragile X alleles in cell culture should be addressed through this project. Participants expressed appreciation for the efforts of CDC and NIST to develop QC materials needed for standardizing Fragile X repeat sizing and suggested there might be a need to revalidate fragile X testing protocols once the QC standards are available.

QC Material Needs for Other Testing Areas

Participants suggested that QC material needs for biochemical genetic and cytogenetic tests should also be addressed. It was agreed that the GTQC Program should focus on DNA-based genetic tests initially and later expand into other genetic testing areas. Among the needs suggested for other genetic tests include:

- Recommended population or subpopulation screening tests, such as the ACOG-recommended carrier screening panel for individuals of Ashkenazi Jewish descent. In light of the upcoming CAP proficiency survey and ACMG technical guidelines, appropriate QC materials would be needed for mutations associated with Tay-Sachs disease, Canavan disease, cystic fibrosis, familial dysautonomia, mucolipidosis IV, Niemann-Pick disease type A, Fanconi anemia group C, Bloom syndrome, and Gaucher disease.
- Hemoglobin alleles associated with thalassemias.
- Pharmacogenetic testing, such as polymorphisms in the cytochrome P450 genes associated with drug metabolism.
- QC materials for standardizing trinucleotide repeat sizing for Huntington disease, spinocerebellar ataxia type 1 and type 7, and myotonic dystrophy.
- Cell lines containing mutations for disorders detected in newborn screening tests such as phenylketonuria and medium-chain acyl-CoA dehydrogenase deficiency
- QC materials for cancer genetic tests for diagnostic and prognostic purposes.

Summary and Next Steps

Dr. Boone expressed acknowledgment to all participants, sponsors, and meeting organizers for making this meeting a success. The meeting concluded with the following major outcomes and the next steps:

- The well-received GTQC Program (GTQC) will go forward as a mechanism to assist the genetics community in obtaining appropriate QC materials and to facilitate information exchange and communication for QC material development, contribution, verification, and distribution. The GTQC website will be publicly launched as early as March 2005. This program will focus on DNA-based genetic tests initially and phase in coordination efforts to address and improve availability of QC materials for other testing areas, including biochemical genetic tests, certain molecular cytogenetic tests, and molecular oncology tests.
- CDC will coordinate teleconferences with the Expert Panel, to further discuss how the Panel will provide input and advice to the GTQC Program.
- The Fragile X Working Group will assist with the CDC-Emory collaborative project, including helping to define needs, develop strategies, and participate in verification activities.
- Logistical issues, in particular informed consent issues and the role of IRBs, should be addressed in order to facilitate contribution and public availability of

materials for QC purposes. Experts were identified at the meeting to look into these issues and to strategize approaches.

- NIST will expand activities to develop SRMs for other trinucleotide expansion diseases (in addition to fragile X), including HD, SCAs, DM, and others..
- The next meeting was proposed to be in 12 months, to be co-hosted by CDC and NIST. The location for this meeting will be decided pending additional input.